REMARKS

I. Status of the claims

Claims 15-39 are pending. Claims 1-14 were previously canceled without prejudice or disclaimer. No claim has been added. Claims 15, 24-26, 29, 31, and 37 have been amended for the following reasons.

The denoted claims have been amended to qualify the type of radiation to which the subject with cancer is exposed. Specifically, the claims have been amended to recite that the radiation employed is "external" radiation. By this, Applicants mean radiation that is administered to the subject from an external radiation source. This is in contrast to "internal" radiation, which speaks to the ingestion or injection of a radioisotope into the subject. "External" radiation is fully supported by the specification. See, for instance, paragraph 13 at page 6, and paragraph 158 at page 77.

II. Information Disclosure Statement

The Examiner states that the non-U.S. patent documents listed in Applicants' IDS of April 19, 2004, were not found in the parent application, USSN 10/300,031. Accordingly, the Applicants resubmit those references (Attachments A-C).

III. Priority

Pursuant to the Examiner's request, Applicants have replaced the first paragraph of the application to specifically reference the patented status of the ancestral applications to which the present case claims priority.

IV. Claim 31 has been amended to correct antecedent basis

Applicants thank the Examiner for pointing out that "Gray," as recited in claim 31, does not have antecedent basis. Accordingly, Applicants have amended claim 31 to recite "Gy" radiation units, which is consistent with claim 30 from which it depends. Applicants request that this objection be withdrawn.

V. There is no statutory double-patenting

Claims 15-39 are rejected under 35 U.S.C. § 101 as claiming the same invention as that of claims 1-25 of U.S. Patent No. 6,730,699. This is a double-patenting rejection.

The present claims are drawn to an invention, namely the administration of "external" radiation to a subject who has cancer, which is not identical to the claims of the '699 patent. Accordingly, there is no basis for this "same invention" rejection and Applicants respectfully request that the Examiner withdraw this rejection.

VI. Overcoming the obviousness-style double-patenting rejection

Claims 15-39 are rejected under the judicially created doctrine of obviousness-style double-patenting rejection as allegedly unpatentable over claims 1-25 of U.S. Patent Nos. 6,515,017 in view of 6,441,025, and over claims 1-20 of U.S. Patent No. 6,441,025.

Applicants will offer to file a terminal disclaimer pursuant to 37 CFR 1.321(c) once they receive the Office's next consideration regarding the allowability of the presently amended claim set.

VII. Conclusion

Applicants believe that the present application is now in condition for allowance. The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

Respectfully submitted,

Date 21 January 2005

By S. A. Bent

FOLEY & LARDNER LLP
Customer Number: 22428
Telephone: (202) 672-5404
Facsimile: (202) 672-5399

Stephen A. Bent
Attorney for Applicants
Registration No. 29,768

A9

cti

9th Annual International Symposium on Recent Advances in Drug Delivery Systems

February 22 – 25, 1999 Salt Lake City, UT

ENHANCEMENT OF TUMOR RADIORESPONSE OF A MURINE OVARIAN CARCINOMA BY POLY(L-GLUTAMIC ACID)-PACLITAXEL CONJUGATE

C. Li, S. Ke, E. Oldham, L. Milas, N.R. Hunter, W. Tansey, C. Charnsagavej, S. Wallace
The University of Texas M. D. Anderson Cancer Center
1515 Holcombe Boulevard, Houston, Texas 77030

INTRODUCTION

The combination of chemotherapy and radiation therapy in the treatment of a variety of tumors has produced substantial improvement in complete response and survival rates (1). Both in vitro and in vivo studies have demonstrated that paclitaxel can strongly enhance tumor radioresponse. In animal studies, the enhancement factors range from 1.2 to more than 2.0, depending on the tumor type, drug concentration, and dose scheduling (2). Since macromolecular chemotherapeutic agents demonstrate enhanced permeation and retention (EPR) effects in solid tumors, it is hypothesized that conjugation of paclitaxel to an appropriate water-soluble polymer would offer increased concentration and duration of tumor exposure to paclitaxel, and thus enhanced interactions of paclitaxel with radiation. In this study, we investigated the radiosensitization effects of poly (L-glutamic acid)-paclitaxel (PG-TXL), a highly efficacious water-soluble paclitaxel conjugate recently developed in our laboratory (3).

EXPERIMENTAL METHODS

PG-TXL was synthesized as described before from of poly (L-glutamic acid) (Sigma, viscosity molecular weight: 31K) (3). The conjugate contained 20% paclitaxel (w/w) which was coupled to PG via ester linkages. Female C3Hf/Kam mice were inoculated i.m. in the right hind leg with 5 x 105 ovarian OCa-1 carcinoma cells. When tumors reached 8 mm in diameter, mice were randomly divided into 12 groups with each group consisted of 6-12 mice. Mice in groups 1-5 were given saline, 14 Gy irradiation alone, or PG-TXL alone at doses of 47, 80, or 120 mg eq. paclitaxel /kg. Mice in groups 6-9 were given PG-TXL at 47 mg eq. paclitaxel/kg and 14 Gy local irradiation at 2, 24, 48, and 144 h after PG-TXL treatments. Group 10 was given PG-TXL at 80 mg eq. paclitaxel/kg and 14 Gy at 24 h after PG-TXL treatment. Groups 11 and 12 were given PG-TXL at 120 mg eq. paclitaxel/kg and 14 Gy at 24 h prior or 24 h after PG-TXL treatment. PG-TXL were given in a single intravenous injection. Local gamma irradiation to the tumor were delivered from a 137Cs irradiator at a dose rate of 7 Gy per minute. Tumor growth delay was determined by measuring three orthogonal tumor diameters until tumors reached 14 mm in diameter.

RESULTS AND DISCUSSION

The radiosensitization effects of PG-TXL were dose dependent. At lower PG-TXL dose of 47 mg eq. paclitaxel/kg, subadditive effect was observed. The mean enhancement factors varied from 0.54 to 0.75 depending on the timing of radiation delivery. However, superadditive effect was observed at higher doses of PG-TXL. The mean enhancement factors increased from 0.75 to 1.8 and 4.2 when PG-TXL was given at 24 h prior to irradiation and PG-TXL doses were increased from 47 to 80 and 120 mg eq. paclitaxel/kg (Table 1). The subadditive effect of chemoradiation observed with PG-TXL at lower dose may be attributed to inadequate cell killing and rapid repopulation of surviving cells. At higher doses, PG-TXL may have profound effects on population of cycling tumor cells and/or on tumor reoxygenation, resulting in significantly enhanced radiosensitization effect. Interestingly, when tumors were irradiated at 14 Gy prior to treatment with PG-TXL at 120 mg eq. paclitaxel/kg, superadditive effect with enhancement factor of 4.3 was observed (Table 1). This is in contrast with previous observation that paclitaxel induces radiation resistance when it was given after irradiation (4).

Table 1 Effect of PG-TXL on radioresponse of murine ovarian OCa-1 tumor

Treatments	Radiation (14 Gy)	Days for tumor to grow from 8-14 mm (mean±SD)	Absolute growth delay in days[a]	Normalized growth delay (mean ± SD)[b]	Enhancement Factors (95% C.I.)[c]
Saline	No	17.2 ± 2.2			
PG-TXL 47 mg eq./kg	No	19.8 ± 0.98	2.7		
PG-TXL 80 mg eq./kg	No	25.3 ± 3.9	8.1		
PG-TXL 120 mg eq./kg	No	29.7 ± 3.2	12.5		
14 Gy radiation alone	Yes	37.9 ± 6.1	20.7		
PG-TXL 47 mg eq./kg	Yes	35.3 ± 4.7	18.2	15.5 ± 4.7	0.75 (0.5-0.98)
PG-TXL 80 mg eq./kg	Yes	62 ± 4.6	45.6	37.5 ± 4.6	1.8 (1.5 - 2.2)
PG-TXL 120 mg eq./kg	Yes	115 ± 3	98.4	85.9 ± 3.0	4.2 (3.9 - 4.3)[e]
PG-TXL 120 mg eq./kg	Yes[d]	117 ± 1.2	100.5	88 ± 1.2	4.3 (4.1 - 4.4)[e]

a. Absolute growth delay is defined as the time in days for tumors in treated groups to grow from 8 to 14 mm minus the time in days for tumors in saline treated group to grow from 8 to 14 mm.
b. Normalized growth delay is defined as the time in days for tumors to grow from 8 to 14 mm in mice treated with the combination of PG-TXL and radiation minus the time in days for tumors to grow from 8 to 14 mm in mice treated with PG-TXL alone.
c. Enhancement factors is obtained by dividing normalized tumor growth delay in mice treated with PG-TXL plus radiation by the absolute growth delay in mice treated with radiation alone.
d. Radiation was given at 24 h prior to PG-TXL treatment. All other combination groups: radiation was given at 24 h after PG-TXL treatment.
e. Data based on tumors that had regrown on day 120. Tumors in 2 out of 6 mice for both groups were still not measurable on day 120.

CONCLUSION

The results of this study suggest that PG-TXL may be effectively used in combination with radiotherapy either before or after irradiation, and that conjugation of radiosensitizers to water-soluble polymeric carriers may offer enhanced radiosensitization effect.

REFERENCES

1. M.Z. Rotman, Radiology, 184: 319-327 (1992).
2. L. Milas, M. Milas and K.A. Mason, Sem. Oncol., submitted (1998).
3. C. Li, D.-F. Yu, R.A. Newman, F. Cabral, L.C. Stephens, N. Hunter, L. Milas and S. Wallace, Cancer Res., 58: 2404-2409 (1998).
4. M.L. Ingram and J.L. Redpath, Int. J. Radiation Oncology Biol. Phys., 37: 1139-1144 (1997).

Acknowledgment
Supported in part by NCI grant R29CA74819, by M.D. Anderson Cancer Center Breast and Ovarian Cancer Research Programs, and by Gianturco Fund and Dunn Foundation.

A10

REF # 248

cti
Discovery • Treatment • Recovery

PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH

VOL 40 #4223 MARCH 1999

#4223 Schedule-independent radiosensitization of a murine ovarian OCa-1 tumor by PG-TXL. Ke, S., Oldham, E., Milas, L., Hunter, N.R., Tansey, W., Charnsagavej, C., Wallace, S. and Li, C. *M.D. Anderson Cancer Center, Houston, Texas 77030.*

Studies have demonstrated that paclitaxel radiosensitizes tumor cells both in vitro and in vivo. However, subadditive interaction was observed when radiation was given prior to paclitaxel treatment. We investigated the radiosensitization effects of PG-TXL, a highly efficacious water-soluble poly (L-glutamic acid)-paclitaxel conjugate. Female C3Hf/Kam mice were inoculated i.m. with OCa-1 tumor. When tumors reached 8 mm in diameter, PG-TXL at equivalent paclitaxel doses of 47 to 120 mg/kg were given i.v. at various times prior to or after 14 Gy local irradiation. Tumor growth delay was determined by measuring three orthogonal tumor diameters until tumors reached 14 mm in diameter. The radiosensitization effects of PG-TXL was dose dependent. The mean enhancement factors were 0.75, 1.8, and 4.2 when irradiation was given at 24 h after treatment with PG-TXL at equivalent paclitaxel doses of 47, 80, and 120 mg/kg, respectively. However, when tumors were irradiated at 14 Gy prior to PG-TXL at 120 mg/kg, the enhancement factor was 4.3. Therefore PG-TXL at 120 mg/kg had superadditive radiosensitization effect independent of treatment schedule. These results suggest that PG-TXL may be effectively used in combination with radiotherapy either before or after irradiation. R29CA7481901, NCI.

PDD 7541

WATER-SOLUBLE POLYGLUTAMIC ACID-PACLITAXEL CONJUGATE (PGA-PACLITAXEL): ANTITUMOR REGRESSION IN RATS BRAEING 13762 MAMMARY CARCINOMA. Chun Li*, Dong-Fang Yu, Robert A. Newman**, and Sidney Wallace. Department of Diagnostic Radiology and **Clinical Investigation, Univeristy of Texas M. D.Anderson Cancer Center, Houston, Texas 77030

Paclitaxel (Taxol), a poorly soluble plant product, is now considered an important, active anticancer drug against a wide variety of solid tumors. Because of its low solubility, it is formulated with cremophor, a vehicle that may attribute to undesirable toxicity. We now report the synthesis and evaluation of water-soluble PGA-paclitaxel. This polymer conjugate has high water solubility (>20 mg/ml) with good stability. The $t_{1/2}$ and t_{90} of the conjugate in phosphate buffered saline at 37 °C were 7 days and 1 day respectively. The PGA-paclitaxel conjugate inhibited the growth of MCF7 and 13762 mammary carcinoma cells *in vitro* to an extent similar to that of paclitaxel. In rats bearing 13762 mammary tumor (approx. 2000 mm^3 at time of treatment), a single intravenous injection of the polymer conjugate (40 mg equiv. paclitaxel/kg body weight) induced complete tumor regression. In comparision, paclitaxel produced a tumor gowth delay of only 9 days (vs. vehicle treated controls). Inhibition of tumor growth produced by PGA-paclitaxel was achieved with less toxicity (body weight loss) than that produced by the less effective paclitaxel treatment. These data suggest that PGA is a good water solubilizing material for paclitaxel and that the PGA-paclitaxel conjugate can produce superior antitumor efficacy with reduced toxicity.

PDD 7542

REHYDRATION KINETICS OF CYTOSINE: CHARACTERIZATION BY WATER VAPOR SORPTION ANALYSIS AND X-RAY POWDER DIFFRACTION. Vidya Joshi,* Ralph R. Pfeiffer, Joseph G. Stowell and Stephen R. Byrn. Department of Medicinal Chemistry and Chemical Pharmacology, Purdue University, West Lafayette, IN 47907-1333

A large number of pharmaceutically active solid drugs occur in the form of stoichiometric hydrates. Pharmaceutical solids may come in contact with water during processing steps such as crystallization, lyophilization, wet granulation, aqueous film coating, and spray drying, as well as from exposure to different relative humidities. The physicochemical and bioavailability properties of these drug hydrates are quite specific to their level of hydration. Hence, it becomes important to have an in-depth understanding of the rehydration behavior of anhydrous pharmaceuticals that can also exist as hydrates. Cytosine, one of the bases in DNA and RNA, was chosen as the model compound to study and characterize the kinetics and mechanism of a solid–state rehydration reaction. The hydration kinetics were monitored using water vapor sorption analysis and X–ray powder diffraction (XRPD). The effect of parameters such as water activity, grinding, and nucleation (seeding) were studied. Cytosine samples were monitored in triplicate for weight gain and changes in X-ray powder diffraction patterns along the course of the hydration reaction. Both the water vapor sorption and X-ray diffraction studies showed that the seeded and ground samples were the fastest to hydrate. The dominance of seeding or grinding in accelerating the reaction rate is dependent on the relative humidity. Statistical evaluation of the fraction reacted(α)-time(t) data from both the studies correlate well, suggesting that the solid-state rehydration of cytosine predominantly proceeds by a mechanism controlled by advancement of the reactant-product phase boundary.

PDD 7543

SOLID-STATE DEGRADATION OF THE MODEL *N*-CARBOXYALKYL DIPEPTIDE ACE INHIBITORS: THE EFFECT OF MOLECULAR MOBILITY AND WATER. Wei Xu*, Joseph G. Stowell and Stephen R. Byrn. Department of Medicinal Chemistry, Purdue University, West Lafayette, IN 47907-1333

Chemical stability of solid pharmaceuticals are often affected by their physical properties. Drugs may exist in crystalline and amorphous forms which are believed to have different molecular mobility. The goal of this project is to study the influence of molecular mobility on solid-state reactivity. The *N*-carboxyalkyl dipeptide ACE inhibitors are not stable in the solid state. The major degradation pathway involves an intramolecular cyclization which requires some extent of molecular reorientation. Two model compounds, Spirapril Hydrochloride and Quinapril Hydrochloride were chosen for the study. The cyclization rates of the crystalline and amorphous forms of the model compounds at elevated temperatures (70-100 °C) and 0.5% RH were measured by HPLC . Results showed that cyclization occurred faster in the amorphous forms and the activation energy of the reaction was also lower in the amorphous forms. High resolution solid-state NMR was used to explore the molecular dynamics of the model compounds. A combination of several pulse techniques indicated that the amorphous samples had greater molecular mobility than their crystalline counterparts. A correlation between chemical reactivity and molecular mobility is implicated by this study. The effect of water on the solid-state cyclization was also investigated. Water lowered the glass transition temperature of the amorphous drugs and caused the cyclization rate to increase. It suggests that water can affect a solid state reaction by altering the molecular mobility.

PDD 7544

DEHYDRATION BEHAVIOR OF NEDOCROMIL MAGNESIUM PENTAHYDRATE Haijian Zhu[1]*, Brian E. Padden[2], Eric J. Munson[2] and David J.W. Grant[1]. Departments of Pharmaceutics[1] and Chemistry[2], University of Minnesota, Minneapolis, MN 55455

Knowledge of the dehydration behavior of drug hydrates is relevant to pharmaceutical formulation, because the physicochemical, biological and mechanical characteristics of hydrates differ from those of the anhydrate, as a result of differences in crystal structure. Nedocromil magnesium (NM), a bivalent metal salt form of nedocromil sodium used in the treatment of reversible obstructive airways diseases such as asthma, exists in several hydration states. Among them, NM pentahydrate is the thermodynamically stable form under ambient conditions. Its dehydration proceeds by the following steps: NM pentahydrate -> NM monohydrate -> NM anhydrate. The kinetics and activation energies of the dehydrations were determined by isothermal TGA and Kissinger's method. The first dehydration step followed a zero order rate equation, suggesting a one dimensional phase boundary controlled mechanism, while the second dehydration step followed the Prout-Tompkins equation, suggesting a nucleation controlled mechanism. Variable temperature powder X-ray diffraction, ^{13}C solid state NMR and hot stage microscopy were used to provide insight into the dehydration mechanisms and to probe any solid phase transformations during the dehydration processes. A correlation was established between the dehydration behavior and the bonding environment of the water molecules in the crystal structure. We thank Fisons plc, Pharmaceutical Division (now Asta Charnwood), Loughborogh, UK, for financial support.

September 1996 (Supplement)
Volume 13, Number 9
PHREEB 13(9) S1–S604 (1996)
ISSN 0724-8741

PHARMACEUTICAL RESEARCH

An Official Journal of the American Association of Pharmaceutical Scientists

Encompassing Research in the Physical, Chemical, Biological, Clinical, Socioeconomic, and Pharmacoeconomic Aspects of the Pharmaceutical Sciences

aaps

PLENUM PRESS • NEW YORK AND LONDON